

REMARKS

Claims 128-145 are cancelled herein and it is respectfully requested that claims 146-161 be entered. The first page of the present application incorporates by reference U.S. Patent Application Serial No. 08/027,146 (the '146 application), which was filed 5 March 1993. A substitute specification is submitted herewith as Exhibit A which incorporates the text from the '146 application. A marked version of the substitute specification is attached herewith as Exhibit B. No new matter has been added. In addition, a copy of the '146 application as filed is also attached for reference. Citations to the text below refer to the '146 application.

The new claims are fully described in the application. The '146 application as filed describes a process in which potential epitopes are identified by scanning the amino acid sequence of an antigen using a motif and then synthesizing the epitope sequence on page 10, lines 23-26. Also, nucleotide sequences which encode peptides comprising an HLA-A2.1 epitope are described on page 19, line 17 to page 20, line 13. The specification also describes methods for testing whether peptide fragments bind an HLA-A2.1 molecule and induce a CTL on page 36, line 1 to page 38, line 24 and on page 72, line 35 to page 76, line 30. Peptide epitopes consisting of 9 to 10 amino acids are described throughout the specification, for example, on page 4, lines 31-36. Peptide fragments consisting of 9 to 10 amino acids are described on page 3, line 4 and line 33, and peptides having a length of less than 15 amino acids are described on page 3, lines 31-32. Amino acid anchors at position 2 and the carboxyl terminus of the epitope are set forth in Table 5 on page 42; L, M, V, I, T and A are all tolerated at position 2 (see also page 39, line 29) and V, L, I, A and M are tolerated at the C-terminus (see also, page 39, line 34). The application also discloses that certain amino acids are preferably not permitted in positions 1, 3, 6 and 7 in instances where the binding motif is a 9-mer and certain amino acids are preferred in positions 1, 3, 4, 5 and 7 of these 9-mers. These data are shown in Table 8 on page 48. They form the basis for claims 147-148 and 156-157. Similarly, amino acids which are preferably not permitted in positions 1, 3-5, and 7-9 of 10-mers, and amino acids that are preferred in positions 1, 3, 4, 6 and 8 of 10-mers are set forth in Table 13 on page 55.

This table forms the basis for claims 86-87 and 96-97. Support for claims 152-153 is found on page 10, line 32-page 11, line 9. Support for the particular peptide in claim 161 is found in the present application (not the '146 application) in Table 3 on page 41. Claim 160 is consistent with the support for tolerated amino acids at anchor positions 2 and the C-terminus in 9-mers and 10-mers but does not include any coincidentally disclosed peptides of which applicants are aware.

Thus, the claims do not add new matter as they find a full description in present application, including the '146 specification filed 5 March 1993.

Applicants appreciate that considerable time and effort has been expended in arriving at the appropriate subject matter to be examined. It is believed that the proposed claims are consistent with the restriction requirement. Election between claims drawn to peptides and claims drawn to nucleic acids was required. The claims as presently drawn relate to peptides.

The requirement for a species election is requested to be withdrawn in view of the present submission. The claims do not relate to a different invention, and it is believed that prosecution is advanced by focussing on the appropriate aspects of the invention. Linkage to a T helper epitope is no longer included in the claims; any particular peptide species misses the point of the invention. The consideration of the Examiner in reviewing the explanation of the invention set forth below and in examining the claims as presented is respectfully requested.

The Invention

The invention provides at least two advances in the art of designing immunogenic peptides and the nucleic acids encoding them. The application is based on an article published by the inventors subsequent to the filing of the '146 application, Ruppert, J., *et al.*, *Cell* (1993) 74:929-937. A copy is enclosed as Exhibit C. First, the invention expands the possible candidates by identifying additional residues that are tolerated in the primary anchor positions at position 2 and the C-terminus of the immunogenic peptide. Previous studies by Falk, *et al.*, *Nature* (1991) 351:290-296 and by Hunt, *et al.*, *Science* (1992) 255:1261-1266 had identified an

HLA-A2.1 motif defined as L and M in position 2 and L, V or I in position 9. The inventors have demonstrated, as shown in the specification, that in addition, immunogenic peptides may include I, V, A or T in the 2 position and may include A and M at the C-terminus. Claims 146 and 154 are directed to taking advantage of these newly discovered motifs; it will be noted that in these claims, either A or M must be at the C-terminus or I, V, A or T must be at the 2 position. The specific peptides of claim 160 have been verified to be immunogenic.

In addition, the invention has contributed an understanding of a way to enhance the predictability of the immunogenicity of the peptides by recognizing the importance of, and identifying the nature of, the secondary anchors. This aspect is claimed in dependent claims 147-151 and 156-158.

Thus, additional immunogenic peptides which would not have been contemplated from the knowledge of the art have been found. This is verified in the declaration of Dr. Sette attached hereto. In addition, the amount of routine screening needed to verify immunogenicity has been diminished by the identification of the secondary residues.

Claim 160 is directed to peptides of less than 15 amino acids which are described by the newly discovered primary anchor generated motifs; claim 161 claims a specific peptide.

The following comments address the specific rejections and objections raised by the Office.

Rejection of Claims Under 35 U.S.C. §112, First Paragraph, for Alleged Lack of Written Description Support in the Specification

A number of claims were rejected under 35 U.S.C. §112, first paragraph as the specification allegedly did not provide an adequate written description for those claims. It is submitted that the rejection is not applicable to the presently submitted claims. Location in the application of this support is described above. Furthermore, the new claims lack the terms objected to in the Office action. For example, the new claims do not include the term "epitope

consisting of about 8-11 amino acids" and "Pan DR" epitope. Thus, it is submitted that the new claims find a written description in the application.

Rejection of Claims Under 35 U.S.C. §112, First Paragraph, for Alleged Lack of Possession of the Invention

Claims 128,129,137, and 145 were rejected under 35 U.S.C. §112, first paragraph, as the Office asserts that the subject matter was not described so as reasonably to convey to own skilled in the art that the inventors had possession of the invention - *i.e.*, that although the claimed peptides were described as "immunogenic" in reality there is no assurance that they are. The Office asserts that it would take "undue experimentation" to determine which peptides encompassed by the formulas in the claims actually are immunogenic. It is this point that applicants dispute.

The Office bases its view on several cited documents. Celis is cited as teaching that an immunogenicity assay *per se* is needed to establish peptide immunogenicity because besides MHC binding, other factors such as antigen processing, peptide transport and the composition of the T cell repertoire could determine whether the peptides can be effective as CTL antigens. Rammansee is quoted as teaching the inadequacy of MHC peptide binding assays and Ochoa-Garay, similarly is said to state that variables such as CTL precursor frequency, peptide hydrophobicity and stability can influence even the *in vitro* induction of CTL responses.

There is no question that not all peptides which bind to an MHC Class I antigen will elicit a CTL response restricted by that Class I antigen and that additional assays would be required to verify immunogenicity. The point is that such verification is routine and was routine at the time the application from which priority was claimed was filed. The disclosures of Celis, Ochoa-Garay, and Rammansee do not contradict this. Indeed, Celis is supportive of the points made here by stating on page 1424:

Several observations support the notion that the immunogenic potential of a peptide is directly correlated with the peptide's binding affinity to the MHC molecule (citations omitted).

Celis further states:

The HLA-A motifs mentioned above have been recently validated by showing that using these motifs, one can predict the majority of the peptides that exhibit a significant binding potential to its respective MHC molecule. (Citation omitted.)

Thus, the Celis document itself supports the nexus between immunogenic character and HLA binding and the nexus between a particular motif and HLA binding.

The Sette Declaration, provided herewith, demonstrates that as of March 1993, only routine experimentation would be required to identify immunogenic peptides having an HLA-A2.1 motif since methods for screening any particular peptide for CTL induction and recognition were well known and routinely practiced. In addition, it was understood that it would not be expected that every single peptide tested would prove to be immunogenic and this is considered acceptable. This is also consistent with the legal standard enunciated by *In re Wands*, discussed below, which specifically takes account of the "Foreman factors" alluded to by the Office in the citation of *Ex parte Foreman*. Thus, the specification enables the methods of claims 146-153 and 154-159 as well as the peptides of claims 160-161 without requiring undue experimentation.

In addition, it has been established that 9-mers and 10-mers lacking the expanded motif described herein have zero possibility of binding HLA-A2.1 molecules. This is shown, for example, in figure 1 of the Ruppert, *et al.*, article attached hereto. Thus, by confining further experimentation to peptides comprising the expanded motif, considerable screening, albeit routine screening, can be avoided.

As further discussed below, the Board of Patent Appeals and the Court of Appeals for the Federal Circuit have articulated a standard for enablement which does not require a high degree of predictability when routine screening methods are available. Thus, it is clear that the teachings of the specification and the teachings in the art were sufficient for allowing the skilled artisan to routinely practice the claimed subject matter as of March 1993.

A. Declaration of Alessandro Sette

Applicants request that the Examiner refer to the Declaration by Alessandro Sette for the following discussion. Attached to Dr. Sette's declaration is a table showing later published results employing the expanded motif. The documents set forth in the table are submitted along with a PTO 1449 form listing them. Also listed on the PTO 1449 form and submitted with it are the papers listed in paragraph 2 of Dr. Sette's declaration.

The Sette Declaration explains that numerous publications predating the March 1993 effective filing date describe the routine use of both HLA binding assays for identifying potential immunogens (paragraph 6), as well as *in vitro* and *in vivo* methods for confirming whether a peptide is immunogenic or not immunogenic (paragraphs 4 and 5). Exemplary studies in which researchers routinely utilized these assays are described in the declaration in paragraphs 7 and 8.

The declaration also demonstrates that the motif itself reduces the amount of experimentation required to determine whether a peptide is immunogenic. Knowledge of a particular motif, such as the HLA-A2.1 motifs described in the specification, can reduce the number of peptides required for testing in immunogenicity assays by 10.8-fold (paragraph 9). Furthermore, the declaration makes it clear that the skilled artisan was prepared as of March 1993 to screen multiple peptides to determine which of those were immunogenic (paragraph 12, referring to Hill), and that the procedures utilized by the skilled artisan before the filing date of the application are similar to those described in the application (paragraph 13). The approach of using the tolerated motifs to screen for and confirm immunogenic peptides was repeatedly confirmed subsequent to March of 1993 as shown in paragraphs 15-16.

Moreover, because the peptides described in paragraph 11 of the declaration were recognized by CTLs from infected patients, immunized individuals, and naturally exposed individuals, the factors noted by the Office as being potentially problematic are not major obstacles to practicing the claimed subject matter (paragraph 17).

Thus, the Sette declaration demonstrates that the claimed methods can be practiced without undue experimentation due to the predictive value of the HLA-A2.1 motif and the routine application of screening procedures taught in the '146 specification.

B. Post-Filing Evidence that the Claimed Subject Matter is Enabled

Paragraphs 15-16 of the Sette declaration describe studies performed after March 1993 in which researchers utilized the HLA-A2.1 motifs and methods disclosed in the specification to identify immunogenic peptides. As can be seen in the table attached to the declaration, many researchers utilized these motifs to identify multiple immunogenic epitopes from a wide variety of antigens. It is therefore clear that the methods set forth in the specification and claimed herein could be routinely practiced by the skilled artisan without undue experimentation at the time the application was filed.

C. Peptide Processing and Peptide Length Are Not Significant Obstacles to Practicing the Claimed Subject Matter

As noted above, paragraph 17 in the Sette declaration establishes that antigen processing and other factors mentioned in the Office action are not significant obstacles to practicing the claimed subject matter. In addition, other studies provide further evidence that these factors are not a significant barriers to practicing the claimed subject matter.

CTL responses are induced as a consequence of naïve T cells recognizing a complex between antigenic peptides and class I molecules. These peptides, which range in length from 8-11 amino acids, are the result of proteolytic degradation of intact antigen (Niedermann, *et al.*, *Immunol. Rev.* (1999) 172:29-48). These processed peptides then bind to class I molecules and are presented on the cell surface of the cell. While longer fragments of the antigen may be utilized to induce CTL responses, ultimately it is the presentation of the minimal epitope by the corresponding class I molecule that results in the differentiation and expansion of the naïve T cell.

Further, the binding of the minimal epitope is dictated by the sequences within the epitope itself and not the surrounding amino acids (Sette & Sidney, *Curr. Opin. Immunol.* (1998) 10:478-482; Medden, *Ann. Rev. Immunol.* (1995) 13:587), and therefore, the motif set forth in the claimed methods provides for HLA binding and the resulting immune response. In the case of longer peptides, the presence of additional amino acids does prevent complex formation, but these epitopes are typically processed by intracellular proteosomes that recognize cleavage sites adjacent to the minimal epitope (Del Val, *et al.*, *Cell* (1991) 66:1145-1153; Eisenlohr, *et al.*, *J. Exp. Med.* (1992) 175:481-487). Using variable length fragments, it has been demonstrated that efficient epitope processing can occur irrespective of the fragment's length (Niedermann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1996) 93:8572-8577; Niedermann, *et al.*, *Immunol. Rev.* (1999) 172:29-48).

Additionally, epitope processing is not limited exclusively to intracellular proteosomes. Several studies have shown that serum and membrane associated proteases can effectively process longer peptides resulting in the generation of the minimal epitope capable of being presented bound to the corresponding HLA class I molecule (Sherman, *et al.*, *J. Exp. Med.* (1992) 175:1221-1226; Kozlowski, *et al.*, *J. Exp. Med.* (1992) 175:1417-1422). Thus, the presence of additional residues besides the minimal epitope might influence the degree of immunogenicity or antigenicity, defined as the amount of peptide necessary to achieve a given level of response. However, antigen length is not a major variable with regard to practicing the claimed methods in the sense that longer peptides are clearly active in various *in vitro* and *in vivo* models (see for example Kast, *et al.*, *Eur. J. Immunol.* (1993) 23:1189-1192).

Moreover, other studies have demonstrated that multi-epitope polypeptides and minigenes can be effectively used to induce cellular immune responses, in particular by enhancing the efficiency of epitope processing. Binding of the optimal epitopes remains constant. In fact, armed with the knowledge of the certain CTL-inducing epitopes, researchers were able to augment immune responses. For example, Shastri, *et al.*, *J. Immunol.* (1995) 155:4339-4346 and Bergmann, *et al.*, *J. Virol.* (1994) 68:5306-5310 demonstrated increasingly

efficient epitope processing resulting from changing the amino acids that flank an epitope.

Moreover, Ishioka, *et al.*, *J. Immunol.* (1999) 162:3915-3925 demonstrated that it is possible to induce CTL responses *that are stronger* than those induced by an intact antigen when multiple epitopes are delivered as a string of peptides.

These examples further illustrate that antigen processing and other factors do not significantly impact binding between the epitope and the HLA class I molecule or impact the immunogenicity of the component epitopes once generated. Thus, applicants submit that claims 146-150, 154, and 156-161, which for example may require the peptide to be processed from a longer polypeptide before contacting the HLA molecules, are fully enabled.

D. The Rejection is Analogous to the Rejection at Issue in *In re Wands*

The routine screening procedure for identifying CTL-inducing peptides is analogous to a screening procedure that the Federal Circuit deemed did not require undue experimentation. The claims at issue in *In re Wands*, 8 USPQ.2d 1400 (Fed. Cir. 1988) were directed to an assay method that required IgM antibodies having affinity for a specific antigen. At issue in *Wands* was whether screening a large number of antibodies to select those required represented undue experimentation. Because the present rejection raises this precise issue - *i.e.*, whether screening multiple peptides to select those that are immunogenic represents undue experimentation, the holding in *Wands* is believed controlling.

The Court in *Wands* determined that screening multiple hybridomas to select particular monoclonal antibodies did not require undue experimentation because 1) screening procedures were taught in the specification and 2) researchers in the field were prepared to screen a large number of hybridomas. Thus, while these hybridoma screening procedures required a significant amount of experimentation, the procedures were routine and the claims were therefore in accordance with 35 U.S.C. §112, first paragraph.

The *Wands* Court noted that Wands used a commercially available radioimmunoassay kit to screen clones in a preliminary screen which identified a subset of candidates that, in order to

satisfy the limitation of the claims, "require further screening to select those which have an IgM isotype and have a binding affinity constant of at least 10^9 M." The Court noted that the results of the test kit do not provide a numerical affinity constant, which must be measured using the more laborious Schatchard analysis. Similarly here, the specification teaches several stages of testing whereby a particular motif is first selected, binding to the appropriate HLA-A2.1 antigen is used to identify a subset that binds and this subset is used in the slightly more laborious assay for immunogenicity. As demonstrated in the Sette declaration, a person of ordinary skill in the art is prepared to screen a number of peptides that correspond to a motif.

Thus, experimentation for practicing the claimed methods and making the claimed nucleic acids is not undue because (1) the specification teaches motifs and screening procedures for identifying CTL-inducing peptides, (2) the screening procedures can be practiced in a routine manner, and (3) researchers are prepared to use these methods to screen a large group of peptides. The facts here are directly analogous to those in *Wands*.

E. The Rejection is Analogous to the Rejection at Issue in *Ex parte Mark*

The situation in the present application is also analogous to that in *Ex parte Mark*, 12 USPQ.2d 1905 (BPAI 1989). In particular, the reasoning set forth in the present Office action is similar to the Examiner's reasoning in *Mark*. The relevant claims at issue were directed to a method for producing DNA encoding a synthetic mutein of any protein by substituting other amino acids for cysteine, wherein the mutein had the biological activity of the parent protein. The claims were rejected as being non-enabled, based on the prior art disclosure that eight such muteins of two different proteins lacked or were substantially reduced in the biological activity of the parent proteins. The examiner reasoned that:

it would require undue further experimentation to construct . . . the *innumerable muteins* encompassed by the instant claims . . . and to screen the muteins produced for any of those which exhibit biological activity after modification.

Id. at 1906 (emphasis added). Additionally, the examiner asserted that most of the muteins “would be inoperative,” and that there was an established unpredictability as to how many muteins would have to be produced in order to obtain even one biologically active embodiment.

On appeal, the Board reversed the enablement rejection, holding that the claims were enabled because they all required “that the mutein retain the biological activity of the native protein.” *Id.* at 1906-7. The prior art muteins lacking activity were “merely examples of work which is *outside* the claims.” *Id.* at 1907. The Board was persuaded that only routine experimentation was required to determine whether a cysteine substitution or replacement would result in a mutein within the scope of the claims. Importantly, the Board stated:

[t]o the extent that the examiner is concerned that undue experimentation would be required to determine other proteins suitable for use in the present invention, we find . . . that only routine experimentation would be needed for one skilled in the art to practice the claimed invention for a given protein. *The fact that a given protein may not be amenable for use in the present invention* in that the cysteine residues are needed for the biological activity of the protein *does not militate against a conclusion of enablement*. One skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity.

Id. at 1907 (emphasis added).

The Board found persuasive the fact that (a) muteins having activity could be routinely identified through the methods disclosed in the specification and the general knowledge in the art, and (b) the claimed method had successfully identified three proteins for which muteins could be made that had the required activity.

Mark is entirely analogous. Claim 146 requires that the peptide is immunogenic in a subject comprising an HLA-A2.1 molecule and that it binds an HLA-A2.1 molecule; claim 154 has a similar limitation as does claim 160. Thus, as in *Mark*, any inoperative embodiments are “merely examples of work which is outside the claims.” Just as in *Mark*, where “one skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity,” one skilled in the art is

clearly enabled to "perform such work as needed to determine whether" the peptides bearing the required motifs are immunogenic. And just as in *Mark* (and in *Wands*), routine assays are described in the specification. And as in *Mark*, the claimed methods also have successfully identified immunogenic peptides. This is confirmed in the Sette declaration paragraphs 15-16.

F. Conclusion

The Sette Declaration and legal precedent make it clear that the claimed methods and compositions may be practiced without undue experimentation, and the rejection under 35 U.S.C. § 112, first paragraph, may properly be withdrawn.

Rejection Under 35 U.S.C. §112, Second Paragraph

A number of claims were rejected under 35 U.S.C. §112, second paragraph, as the terms "at the carboxyl terminus" and "c-terminal position" in the context of the previous claims were allegedly indefinite. As presented in the new claims, the term "C-terminus" is definite as it is referenced to the subsequence or motif. Accordingly, the rejection under 35 U.S.C. § 112, second paragraph, may be withdrawn.

Rejection Under 35 U.S.C. § 103(a)

Claims 128, 129, and 137 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Boon *et al.*, Cheever *et al.*, or Kubo, *et al.* in view of Sette, *et al.* The new claims are supported in the '146 application which was filed 5 March 1993, so the Sette patent filed 14 September 1993 is not prior art. Thus, on a purely formal basis, the rejection as framed is inapplicable to the new claims.

Furthermore, a *prima facie* case for obvious may not be maintained because the cited documents do not result in the claimed subject matter. Claims 146-161 require the steps of (a) providing an amino acid sequence of an antigen of interest, (b) identifying a specific subsequence within that antigen characterized by the motifs described in step (b), and (c) identifying a fragment of the antigen which contains this subsequence. Neither Boon, Cheever,

nor Kubo carries out this set of steps. In particular, neither document discloses identifying subsequence as required by step (b).

Boon describes cloning experiments leading to the nucleic acid sequence of MAGE-1 (column 5) and particular CTL epitopes known in the art (see for example column 14) which is an entirely different method. There is no consideration of identifying a motif specific for a particular HLA antigen, much less HLA-A2.1. Thus, step (b) of claim 146 is completely missing and therefore is not taught or suggested by Boon.

Similarly, Cheever fails to carry out step (b). Instead, Cheever is concerned with identifying peptides derived from the Her2/Neu oncogene that induce an antibody response. There is no teaching or suggestion of scanning the amino acid sequence of the Her2/Neu oncogene for the motif in step (b).

Furthermore, Kubo does not mention the motif set forth in step (b). While Kubo describes methods for scanning antigens for subsequences which correspond to a particular motif, there is no teaching or suggestion of utilizing the particular motifs set forth in the claims. Thus, a teaching or suggestion of step (b) is also missing from Kubo.

Thus, the cited documents fail to describe a method for identifying a specific subsequence within that antigen characterized by the motifs described in step (b), and also fail to describe the motif required by all of the claims. The cited documents therefore may not establish a basis for a *prima facie* case of obviousness.

Conclusions

Formal rejections have been addressed by amendment, and a rejection for lack of an enabling written description has been shown by the declaration of Dr. Sette to be misplaced. As demonstrated by declaratory evidence, only routine experimentation is required to determine which peptides fall within the scope of the claims. Such routine experimentation is recognized as acceptable by the holdings in *In re Wands* and *Ex parte Mark*. Further, no suggestion of the claims as presently proposed is found in the cited documents. For these reasons, it is believed

that claims 146-161 are in a position for allowance and passage of these claims to issue is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket No. 399632000623.

Respectfully submitted,

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